The effect of phenylglyoxal on *Clostridium sporogenes*

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In general phenylglyoxal (PG) was found to inhibit anaerobic bacteria, with strict anaerobes being more affected than facultatives. The susceptibility of Clostridium sporogenes *vegetative cells to PG varied with the type of medium (20–>10 000 μg ml^{-1} PG), pH (20–>2500 μg ml^{-1} PG), and incubation temperature (625–1250 μg ml^{-1} PG). However, optimum vegetative (20 μg ml^{-1} PG) and spore (<1·0 μg ml^{-1} PG) inhibitions occurred in cooked meat medium for all conditions tested. PG was most active against vegetative cells at pH values >7·5 and at temperatures between 25–30°C. The compound inhibited sporogenesis (156 μg ml^{-1} PG) and replication processes (29 μg ml^{-1} PG), as well as deoxyribonuclease activity (4·28 μg ml^{-1} PG). Increased concentrations of free α-amino acids and sulfhydryl-containing compounds antagonized the activity of PG against vegetative cells. Antigerminative effects appeared to occur optimally at low pH, and the heat resistance of* C. sporogenes *spores was reduced. Furthermore, PG concentrations of 10–0·63 μg ml^{-1} prevented germination of* C. sporogenes *spores in five commercial soups and maintained their organoleptic qualities for 35 days at 30°.*

Introduction

Phenylglyoxal (PG) is one of a large group of compounds known as α-dicarbonyls, which are recognized for their group specific reactivites, industrial, and medicinal applications. In alkaline solution it reacts preferentially with the guanidino group of arginine. Thus, PG and several other related compounds are established arginine antagonists that form adducts between guanido groups of arginine. By virtue of its arginine reactivity, PG has been employed to identify the active sites of enzymes where arginine is important in substrate recognition.

Phenylglyoxal has been shown to interfere with anion transport systems (Wieth et al. 1982, Bjerrum et al. 1983), sulfate exchange in red blood cells (Zaki 1982, Zaki and Julien 1985), intermembrane charge movement (Etter 1990), and nitrate absorption (Dhugga et al. 1988). In the presence of PG, anoxic cells are rendered more sensitive to the lethal effects of X-rays. The compound inhibits photophosphorylation (ATP-*Pi* exchange and Mg^{2+} ATPase activities), and interacts with a number of amino acids and sulfhydryl groups that have been demonstrated to be essential for spore germination and vegetative cell growth.

As to whether PG and other α-dicarbonyls are biostatic or biocidal, only limited studies have been reported. 1,2-cyclohexanedione (CHD) was demonstrated to be effective at 0·001 M against

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Mycobacterium tuberculosis (Bloch et al. 1945); and PG was found to inhibit the sporulation, germination, and nutrient acquisition/utilization processes of *Bacillus cereus* (Ram and Rana 1978, Ram et al. 1979). Several aromatic α-ketoaldehydes were tested for their growth inhibitory effects on bacteria and yeast (Vander Jagt 1975). Phenylglyoxal, *p*-chlorophenylglyoxal, and 2,4,6-thrimethylphenylglyoxal were found to inhibit the growth of *Escherichia coli* and *Saccharomyces cerevisiae*. Additionally, a number of α-dicarbonyl and structurally related compounds were tested as antimicrobials against a variety of foodborne organisms (Jay 1982, Jay et al. 1983). As a group, the compounds were most effective against Gram-negative bacteria. PG was unique among those tested in that it was inhibitory to Gram-positive anaerobes.

Although PG has been demonstrated to be effective in inhibiting various metabolic, sporulation and germinative processes, the literature is lacking concerning information with respect to its effect on anaerobic sporeformers. Consequently, it was the aim of this study to define the spectrum of activity of phenylglyoxal against several anaerobes with emphasis on *C. sporogenes*.

Materials and Methods

Bacteriological media

The following standard bacteriological media were obtained from either DIFCO (Detroit, MI) or BBL Microbiological Systems (Cockeysville, MD), and prepared per supplier instructions: nutrient broth (NB, DIFCO & BBL); cooked meat medium (CMM, DIFCO), thioglycollate broth (TB, DIFCO); synthetic-AOAC broth (S-AOAC, DIFCO); deoxyribonuclease agar with Methyl Green (DNase, DIFCO); nutrient gelatin broth (NGB, DIFCO); reinforced clostridial medium (RCM, DIFCO); brain heart infusion broth (BHI, BBL); and trypticase soy broth (TSB, BBL).

Cultures

All cultures were maintained in either thioglycollate broth or cooked meat medium. Prior to use, 48 h cultures were grown at 30°C when vegetative cells were desired. Synchronized spore suspensions were prepared by culturing on reinforced clostridial agar in Gas-Pak jars for 14 days, followed by 14 days aerobic incubation and subsequent harvesting (d-H_2O and centrifugation). Spore pellets were suspended in d-H_2O, heat-shocked (80°C/10 min.) and maintained at 5°C prior to use. Spore suspensions were quantified prior to storage and immediately before antimicrobial testing to determine inoculum concentration. Viability, germination rate, and heat stabilities were tested initially and monthly. The individual bacterial strains employed were: *Clostridium sporogenes* (FDA B459); *C. perfringens* (FDA B4540); *C. sordelli* (laboratory stock culture); *C. bifermentans* (ATCC 19299); *C. difficile* (laboratory stock culture); *Bacteroides fragilis* (ATCC 25285); *Bacillus cereus* (laboratory stock culture); and *Escherichia coli* (clinical isolate). Strict anaerobic cultures were maintained in both TB and CMM, and the facultative and aerobic organisms in CMM.

Minimal inhibitory concentration (MIC) determination

MICs were determined by use of doubling dilutions of PG in various media after autoclaving. Incubation conditions such as atmosphere, temperature, incubation period, and other parameters along with test medium varied and further specifics are noted under the respective protocols.

Phenylglyoxal and other reagents

Phenylglyoxal, diacetyl, and 1,2-cyclohexanedione were obtained from Aldrich Chemicals (Milwaukee, WI). Stock solutions were prepared in ethanol and maintained in amber bottles at 5°C. Gas-Pak system materials (gas generating envelops, palladium catalyst, and indicator strips) were obtained from BBL Microbiological Systems.

Experimental protocol

The following tests were carried out to define the range and parameters of optimal effectiveness of PG against spores and vegetative

cells of *C. sporogenes*. All analyses were conducted at 30°C unless otherwise noted.

Determination of PG vegetative MICs.

(a) PG vegetative MICs were determined for various anaerobes under CO_2-enriched conditions (9·73% CO_2 + 85·27% N_2 + 5% H_2) and in various substrates. Kapak/Scotchpak heat sealable pouches were used for CO_2 atmosphere with a 1·0 min, 20 p.s.i. gasing-out period.

(b) Several shared components among the individual formulations of the media employed were tested to determine their effect on PG against *C. sporogenes*. The compounds tested were: NaCl (0·14 g/100 ml, Fischer), casitone (1·5 g/10 ml, DIFCO), L-cystine (0·05 g/100 ml, Fischer), yeast extract (0·5 g/100 ml, BBL), dextrose (Fisher), sodium-thioglycollate (0·05 g/100 ml, BBL), dipotassium phosphate (0·25 g/100 ml, Fischer), soluble starch (0·1 g/100 ml), Fischer, and NZ amine NAK (17 g/100 ml, Sheffield Chemicals). All possible combinations of the nine components were tested in nutrient broth to determine whether the observed effect was due to synergistic response between two or more components.

(c) The effects of various PG concentrations (5000; 2500; 1250; and 625 $\mu g/ml^{-1}$) on *C. sporogenes* in the presence of six amino acids and sodium thioglycollate were tested at three pH values (6·0, 7·0 and 8·0) in synthetic-AOAC broth at 30°C. The following amino acids were employed at a 1% (w/v) concentration: DL-tryptophan, L(+)-arginine, L(−)-cystine, DL-histidine HCl, L-lysine HCl, and cysteine HCl. Sodium-thioglycollate was tested at 0, 1·0, 2·0, 3·0, 4·0 and 5·0% concentrations (w/v). The test media were inoculated with 0·1 ml of a 48 h culture and incubated anaerobically for 72 h.

(d) The significance of pH (5·5, 6·0 and 8·0) on the observed activity of PG against *C. sporogenes* was tested in NB and S-AOAC containing various PG concentrations (39; 78; 156; 312·5; 625; 1250; 2500; 5000; and 10 000 $\mu g\ ml^{-1}$). Cultures were incubated anaerobically for 48–72 h at 30°C. Furthermore, incubation temperatures of 15, 25, 30, and 45°C and the addition of PG prior to autoclaving on the effects of PG in NB on *C. sporogenes* under anaerobic conditions were tested to determine optimal temperature conditions and the heat stability of the compound.

(e) To assess the effects of PG on sporulation and replication processes of *C. sporogenes*, CMM containing various sublethal PG concentrations (5; 10; 20; 39; 78; 156; 312·5; and 625 $\mu g\ ml^{-1}$) was inoculated with 0·1 ml of a 48 h culture and incubated at 30°C. Three replicate tubes of CMM were used for each of the PG concentrations tested. Methylene blue smears were made at 24, 48, 72 h incubation and four or more microscopic fields examined for the percentage of sporulating/no-sporulating cells, free spores and whether they were phase bright or dark, and the number of replicating bacilli. Attached cells that were in various stages of complete separation, and/or bacilli with distinct invaginations were intepreted as replicating bacilli.

(f) To determine whether or not PG had any effect on deoxyribonuclease and gelatinase activity of *C. sporogenes*, subinhibitory concentrations were added to DNase agar with methyl green and nutrient gelatin broth. A PG dilution series of 0, 0·715, 1·4, 2·8, 4·28, 5·7, 7·12, 8·5, 9·9, 11·3, and 14·2 $\mu g\ ml^{-1}$ was employed for each test medium. The test media were inoculated with 0·1 ml of a heat-shocked spore suspension and incubated anaerobically (Gas-Pak) at 30°C for a period of 48 h. DNase activity was determined by examining colonies for clearing zones formed in the green background of the medium; variations in zone diameters was also noted. Gelatinase activity was assessed by incubating the test tubes at 5°C for 2–4 h and examining for solidification. Negative gelatinase tubes were incubated an additional 48 h and re-examined for enzymatic activity.

Determination of PG spore MICs. A modified version of the Association of Official Analytical Chemists (AOAC) methods of analysis (1984) for sporicidal activity of disinfectants was employed to define the effects of PG on spores of *C. sporogenes*. The parameters tested included substrate conditions, optimum pH, temperature conditions, minimum exposure time, the release of dipicolinic acid (Janssen et al. 1958), thermal resistance, osmotic stresses (added NaCl and sucrose), and preservation of various canned food products. Additionally, diacetyl and 1,2-cyclohexanedione were investigated to assess their effect on *C. sporogenes* spores.

(a) Six culture replicates containing various PG concentrations ranging from 10 000 to 1·0 $\mu g\ ml^{-1}$ were inoculated with 0·1 ml of a heat-shocked spore suspension, and incu-

bated aerobically or anaerobically for exposure periods of 1, 4, 6, 8, 12 and 24 h at 30°C. The test cultures were subsequently heat-shocked and 0·1 ml subcultured to each of five replicate tubes of thioglycollate broth (10 ml) and incubated at 30°C for 48 h.

(b) Release of dipicolinic acid (DPA). The effect of PG on the DPA release was measured using the colorimetric assay of Janssen et al. (1958). One ml of a chromogenic reagent (0·5 M acetate buffer containing 1% $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ plus 1% ascorbic acid) was added to 4·0 ml of a test spore suspension. The color reaction was immediate and was based on the interaction of ferrous iron with DPA with ascorbic acid to increase the stability of the color complex. The reaction was measured spectrophotometrically at 440 nm and DPA content was obtained by use of a calibration curve of known DPA concentrations (0–160 $\mu g\ ml^{-1}$).

(c) Thermal resistance. To assess the effect of PG on the heat stability of *C. sporogenes* spores, CMM containing various PG concentrations were inoculated with 0·1 ml of heat-shocked spore suspension. Test cultures were subjected to an 80°C (water bath) thermal treatment for various time periods (5, 10, 15 and 20 min) and 0·1 ml subcultured to 10 ml tubes of thioglycollate broth. Growth after 48 h at 30°C in the thioglycollate tubes was interpreted as maintenance of heat stability.

(d) Synergistic effects of sucrose and NaCl on PG spore MICs. Various added sucrose and/or NaCl concentrations were tested for their effect on the antigerminative action of PG on *C. sporogenes* spores in CMM at 30°C. CMM with sucrose or NaCl was used as the test medium, and was incubated with 0·1 ml of a heat-shocked spore suspension. One-tenth ml of the exposure medium was transferred to 10 ml tubes of thioglycollate broth prior to and after heat shocking. All tubes were incubated at 30°C for 48 h and examined for turbidity. Treatment effects were interpreted as sporicidal if no growth developed in both heat-shocked and non-heat-shocked inocula. Lack of growth in non-heat-shocked inocula with growth in heat-shocked inocula was interpreted as an antigerminative.

(e) Effect of PG on *C. sporogenes* spores in commercially canned soups. The effect of PG on *C. sporogenes* spores was tested in various canned foods to compare these products as substrates with the observed effects of PG in bacteriological media. The commercially (Campbell Soup Co.) prepared products tested included chicken with rice, French onion, beef noodle, cream of chicken (condensed), New England clam chowder (condensed), and old-fashioned vegetable beef soups. Cooked meat medium was employed as a control. Six 10 ml samples of each soup and CMM were prepared in sterile test tubes and PG was added to effect a dilution series 0·0, 0·625, 1·25, 2·5, 5·0, and 10·0 $\mu g\ ml^{-1}$. The cultures were inoculated with 0·1 ml of a heat-shocked spore suspension (4000 spores ml^{-1}), incubated at 30°C, and observed at 7 day intervals for organoleptic changes induced by growth of the organism. The organoleptic quality of test cultures was assessed by comparing changes in gas production, color, turbidity, consistency, and aroma with those of uninoculated controls.

Results

MIC of PG against vegetative cells

Of the five clostridial species tested, the MIC of PG ranged from 78 to >10 000 $\mu g\ ml^{-1}$ with both *C. sporogenes* and *C. perfringens* being 1250 $\mu g\ ml^{-1}$. *C. difficile* (78 $\mu g\ ml^{-1}$) and *C. sordelli* (625 $\mu g\ ml^{-1}$) were the most sensitive of the Gram-positive anaerobes tested, while *C. bifermentans* (>10 000 $\mu g\ ml^{-1}$) was the most resistant. *Bacteroides fragilis*, a Gram-negative non-sporeforming anaerobe, was inhibited at a concentration of 156 $\mu g\ ml^{-1}$ with 2500 $\mu g\ ml^{-1}$ required to inhibit *Escherichia coli*, a facultative anaerobe. Incubation in a 9·73% CO_2-enriched atmosphere reduced by one half the MIC of PG against vegetative

Table 1. MIC of PG on vegetative cells of *C. sporogenes* in six culture media.

Medium	MIC ($\mu g\ ml^{-1}$)
Nutrient broth	1250
Trpticase soy broth	5000
Brain heart infusion broth	5000
Thioglycollate broth	>10 000
Cooked meat medium	20
Synthetic-AOAC	5000

cells of *C. sporogenes, C. perfringens*, and *C. sordelli*. However, the MIC of PG was eight times lower for *C. bifermentans*, and 16 times higher for *C. difficile*. Vegetative MICs of PG varied widely in different culture media (Table 1) with the value for *C. sporogenes* in thioglycollate medium being > 10 000 μg ml^{-1} while in cooked meat medium (CMM) the MIC was only 20 μg ml^{-1}. In nutrient broth (NB), pH values >7·5 and <6·0 reduced (20 μg ml^{-1}) and increased (2500 μg ml^{-1}) respectively the vegetative MIC of PG. Regarding temperature of incubation, the vegetative MIC of PG against *C. sporogenes* was 625 μg ml^{-1} at 15 and 45°C and 1250 μg ml^{-1} at the other temperatures employed using nutrient broth. When incubated with subinhibitory concentrations of PG, 156 μg ml^{-1} inhibited sporogenesis (Table 2), while the normal distribution of sporulating (27·7%) and non-sporulating (23·5%) cells, and free spores (48·7%) was observed in control cultures. The cell cycle of *C. sporogenes* was limited to vegetative growth by increasing PG concentrations, and 39 μg ml^{-1} reduced the

Table 2. Effect of subinhibitory PG concentrations on cell cycle phases of *C. sporogenes* in cooked meat medium.

PG concentration (μg ml^{-1})	% Cell cycle phase at 30°C after 48 h		
	Sporulating cells	Non-sporulating cells	Free spores
0	27·72 ± 0·7[a]	23·59 ± 1·0	48·65 ± 1·0
5	32·90 ± 0·7	44·24 ± 0·7	22·86 ± 0·6
10	13·41 ± 0·4	28·23 ± 0·4	58·39 ± 0·5
20	11·20 ± 1·0	30·00 ± 0·8	58·80 ± 0·7
39	37·70 ± 0·4	45·71 ± 0·3	16·59 ± 0·5
78	46·22 ± 0·6	44·86 ± 1·0	8·92 ± 0·9
156	0 ± 0·0	65·62 ± 1·0	34·38 ± 1·0
313	0 ± 0·0	98·63 ± 1·0	0·70 ± 1·0
625	0 ± 0·0	99·78 ± 1·0	0·22 ± 0·7

0·1 ml of a heat-shocked spore suspension was used as inoculum. Four microscopic fields of a methylene blue smear for each of three replicate tubes were examined for the percentage of sporulating cells and endospores.

[a]% Standard deviation of variation among individual populations.

Table 3. The effect of three added sulfhydryl-containing compounds and two amino acids on the antimicrobial activity of phenylglyoxal on vegetative cells of *C. sporogenes*.

Additive	48 h MIC (μg ml^{-1}) at 30°C	
	NB	S-AOAC
Control	1250	2500
Sodium thioglycollate	>10 000	312·5
L-cystine	>10 000	>10 000
L(+)-arginine	625	>10 000
Cysteine HCl	>10 000	>10 000
L-lysine	>10 000	156·25

NB, nutrient broth (pH 6·8). S-AOAC w/o Dextrose (pH 6·8). Additives were used at 1% (w/v) concentrations. All tubes were incubated anaerobically.

rate of spore emergence. Vegetative MICs were two times higher in agar than broth medium and were unaffected by the addition of PG prior to autoclaving. Increased concentrations of free amino acids and sulfhydryl containing compounds antagonized the effect of PG on vegetative cells (Table 3). An MIC of 5000–10 000 μg ml^{-1} was found. The MIC of PG in nutrient broth containing added sulfhydryl compounds and L-lysine HCL was eight times greater, while added cystine, cysteine, and arginine doubled the MIC of PG in synthetic-AOAC broth. In the presence of added L(+)-arginine and sodium-thioglycollate, PG MICs were lower in NB and S-AOAC. A PG concentration of 4·28 μg ml^{-1} and higher inactivated deoxyribonuclease activity of *C. sporogenes* while gelatinase activity was unaffected at the concentrations tested. A PG concentration of 8·54 μg ml^{-1} inhibited growth of *C. sporogenes* in DNase agar, with 14·2 μg ml^{-1} and higher being inhibitory in nutrient gelatin broth.

MIC of PG against spores of C. sporogenes

When tested against spores suspensions in four culture media, 0·076 μg ml^{-1} (within 4 h at 30°C) inhibited spore germination in both CMM and thioglycollate medium, with 625 μg ml^{-1} required in NB and 313 μg ml^{-1} required in S-AOAC (Table 4). The inhibition was observed to be independent of pH when tested in NB, S-AOAC, and CMM media that were adjusted to pH values of 6·0, 7·0 and 8·0.

The sporostatic MIC of PG in CMM was increased when tested in the liquid portion of the medium (without pellets) and remained constant at incubation temperatures of 15, 30 and 46°C. Several exposure periods were tested and a minimum time of 3 h was required for germination of *C. sporogenes* at 30°C. A PG concentration of 0·076 μg ml^{-1} was found to delay germination for exposure periods of up to 24 h. As to whether PG affected the release of dipicolinic acid (DPA), concentrations up to 625 μg ml^{-1} had no effect while concentrations above this value had progressive inhibitory effects on the release of DPA.

Table 4. The MIC of PG that prevented spore germination of *C. sporogenes* in four culture media at 30°C.

Test medium	MIC[a] (μg ml^{-1})
Nutrient broth (pH 6·8)	625
Synthetic-AOAC (pH 6·8)	313
Cooked meat medium (pH 7·2)	0·076
Thioglycollate broth (pH 7·2)	0·076

[a]Minimal inhibitory concentrations (MIC) are based on a 95% confidence interval.

Table 5. Effect of PG on *C. sporogenes* in cooked meat medium.

Medium change (30°C)	MIC (μg ml^{-1}) at incubation Time (d)			
	8	21	35	49
Digestion of particles	0·076	156	156	313
Greening of particles	0·076	5	78	78

0·1 ml of a heat-shocked spore suspension was used as inoculum. MIC values represent the PG concentration at which the specified medium change was inhibited. Both particle digestion and greening were observed in control cultures after 8 days of incubation. The percentage of the medium affected increased with time.

Table 6. Minimum quantities of PG that prevented germination of *C. sporogenes* in various commercial soups for up to 35 days.

Test medium	pH	30°C MIC ($\mu g\ ml^{-1}$) Incubation periods (days)				
		7	14	21	28	35
Cooked meat medium	7·2	0·63	1·25	1·25	1·25	1·25
Chicken with rice	6·4	2·5	2·5	2·5	2·5	2·5
French onion	4·7	2·5	2·5	2·5	2·5	2·5
Beef noodle	5·4	10	10	10	10	10
New England clam chowder (condensed)	5·9	0·63	0·63	0·63	0·63	0·63
Vegetable beef old fashioned	5·2	1·25	1·25	1·25	1·25	1·25

4000 spores ml^{-1} were used as inoculum for each test medium. The spore suspension was approximately 2 months old and was prepared in reinforced clostridial medium (RCM). The following phenylglyoxal (PG) concentrations were tested in each product: 10, 5, 2·5, 1·25, 0·625 and 0 $\mu g\ ml^{-1}$.

When tested for its effect on proteolytic activities of *C. sporogenes* in CMM, 78 and 313 $\mu g\ ml^{-1}$ PG delayed, respectively, particle digestion and greening for 49 days (Table 5). Meat particle decay and greening were detectable after 8 days at 30°C in control-CMM (without PG) cultures and increased with timed PG concentrations of 1·25, 2, 5, and 10 $\mu g\ ml^{-1}$ maintained the organoleptic qualities (i.e. color, consistency, aroma, gas production) of beef noodle (pH 5·38), chicken with rice (pH 6·4), French onion (pH 4·67), and vegetable beef (pH 5·2) soups respectively for up to 35 days at 30°C (Table 6).

Table 7. The effect of numbers of spores of *C. sporogenes* on the MIC of PG in cooked meat medium.

Inoculum size (spores ml^{-1})	MIC[a] IC ($\mu g\ ml^{-1}$)
7×10^{10}	>5·0
7×10^{8}	>5·0
7×10^{6}	>5·0
7×10^{4}	0·076
7×10^{2}	0·076
70	0·076

[a]Minimal inhibitory concentrations (MIC) are based on a 95% confidence interval.

The resistance of *C. sporogenes* spores to a heat treatment of 80°C (10 min). was reduced by PG concentrations of 5·0 $\mu g\ ml^{-1}$ or higher. A 50% reduction in the number of surviving spores was observed at PG concentrations of 1·25 $\mu g\ ml^{-1}$, and the rate of reduction was proportional to PG concentration. PG concentrations of 0·076 $\mu g\ ml^{-1}$ and >5·0 $\mu g\ ml^{-1}$ were antigerminative, respectively, against spore concentrations of up to 7×10^4 and 7×10^{10}/ml (Table 7). Added concentrations of sucrose or NaCl were found to enhance the activity of PG against *C. sporogenes* spores in CMM. A concentration of 4·88 $\mu g\ ml^{-1}$ with 0·1% sucrose or 1·0 ml NaCl was sporicidal. Furthermore, the structurally similar compounds diacetyl and 1,2-cyclohexanedione were sporostatic, respectively, at 62·5 and 15·6 $\mu g\ ml^{-1}$ in nutrient broth.

Discussion

Phenylglyoxal (PG) was tested initially for its effect against a variety of anaerobes, most of which were members of the genus *Clostridium*. The findings of this study show that phenylglyoxal (PG) is an effective antimicrobial against the

Gram-positive anaerobes tested. A Gram-negative anaerobe was also inhibited by the compound, while a facultative anaerobe was less affected. When tested against spores of *C. sporogenes*, the compound's activity was dramatically different from those observed for vegetative cells. Less than 1·0 $\mu g\ ml^{-1}$ prevented germination in cooked meat medium, 39 $\mu g\ ml^{-1}$ were required to prevent spore emergence, while 156 $\mu g\ ml^{-1}$ were required to inhibit sporogenesis by vegetative cells. Furthermore, two additional α-dicarbonyl compounds (diacetyl and 1,2-cyclohexanedione) were found to prevent germination. PG's effectiveness was medium dependent, with greater inhibition occurring in cooked meat medium than in nutrient broth. Antigerminative effects appeared to occur optimally in a low pH range, and the organoleptic qualities of various canned soup products were maintained by low concentrations (10·0 to 0·63 $\mu g\ ml^{-1}$) for 35 days at 30°C. The observed delay and/or inhibition of various pre- and post-germination cell phases may be attributed to PG's reactive properties (Takahaski 1968, 1977a,b, Cheung and Fonda 1979, Eun 1988), and the availability of essential nutrients.

These data indicate that phenylglyoxal and perhaps other structurally related compounds may be representative of a new category of antimicrobial chemicals that are effective against anaerobes, and spores of both Gram-positive and -negative bacteria. However, additional analyses are required to determine whether the observed anticlostridial effects are antibotulinal.

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